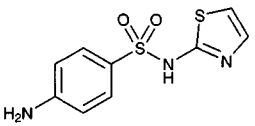
CHROMATOGRAM
Retention time: 6.5 (as the N-propionyl derivative of the metabolite and reduction product, 5-aminosalicylic acid)
Internal standard: N-propionyl-4-amino-2-hydroxybenzoic acid (12.5)
Limit of detection: 100 nM

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
serum; derivatization; SPE

REFERENCE
van Hogezand,R.A.; van Balen,H.C.J.G.; van Schaik,A.; Tangerman,A.; van Hees,P.A.M.; Zwanenburg,B.; van Tongeren,J.H.M. Determination of sodium azodisalicylate, salazosulphapyridine and their metabolites in serum, urine and faeces by high-performance liquid chromatography, *J.Chromatogr.*, **1984**, *305*, 470–476.

Sulfathiazole



Molecular formula: $C_9H_9N_3O_2S_2$
Molecular weight: 255.32
CAS Registry No.: 72-14-0, 144-74-1 (Na salt)
Merck Index: 9115
Lednicer No.: 1 124

SAMPLE
Matrix: blood, milk
Sample preparation: 1 mL Serum or milk + 4 mL MeCN, vortex, centrifuge at 1000 g for 15 min. Remove the supernatant and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 50 μL water, mix vigorously, add 1 mL MeCN, centrifuge at 1000 g for 10 min. Remove the upper layer and evaporate it to dryness, reconstitute the residue in 1 mL 10 ng/mL p-aminobenzoic acid in 0.01% trichloroacetic acid, centrifuge at 1000 g for 10 min. Remove a 500 μL aliquot of the clear layer and add it to 100 μL 1 mg/mL fluorescamine in acetone (prepared fresh each day), mix for 1 min, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm Nova-Pak C18
Mobile phase: MeCN:10 mM KH_2PO_4 30:70
Flow rate: 1
Injection volume: 50
Detector: F ex 390 em 475

CHROMATOGRAM
Retention time: 6.7
Internal standard: p-aminobenzoic acid (5.5)
Limit of detection: 0.1 ng/mL

OTHER SUBSTANCES
Extracted: sulfadiazine, sulfadimethoxine, sulfamethazine, sulfamethoxazole, sulfamonomethoxine

KEY WORDS
cow; serum; derivatization

REFERENCE
Tsai,C.-E.; Kondo,F. Liquid chromatographic determination of fluorescent derivatives of six sulfonamides in bovine serum and milk, *J.AOAC Int.*, **1995**, *78*, 674–678.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30

Detector: UV 200.5

CHROMATOGRAM

Retention time: 9.022

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: eggs, honey, milk

Sample preparation: Honey. Dissolve 1 g honey in 10 mL water, homogenize, filter (0.45 μm), inject a 50 μL aliquot. Milk, eggs. 5 mL Milk or 0.4 g lyophilized eggs + 10 mL trichloroacetic acid solution (so as to give a final trichloroacetic acid concentration of 3%), homogenize, centrifuge at 5000 rpm for 5 min. Re-extract the residue with 10 mL 3% trichloroacetic acid. Combine the aqueous phases and make up to 25 mL with trichloroacetic acid solution, inject a 50 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Spherisorb ODS-2

Mobile phase: Gradient. MeCN:water 3:97 for 5 min, to 40:60 over 15 min, return to initial conditions over 1 min, re-equilibrate for 10 min. (Wash column with MeCN:ethyl acetate 5:95 at the end of each day.)

Flow rate: 1

Injection volume: 50

Detector: UV 260

CHROMATOGRAM

Retention time: 12.5

Limit of detection: 70 ng/mL

OTHER SUBSTANCES

Extracted: sulfadiazine, sulfaguanidine, sulfamethoxazole, sulfapyridine

REFERENCE

Viñas,P.; López Erroz,C.; Hernández Canals,A.; Hernández Córdoba,M. Liquid chromatographic analysis of sulfonamides in foods, *Chromatographia*, **1995**, *40*, 382–386.

SAMPLE

Matrix: eggs, milk, tissue

Sample preparation: Milk. Centrifuge at 2000 g and freeze at -20° to remove the cream. Mix a 5 mL aliquot with 5 mL saline solution and add 1 mL 1% sodium azide solution (Caution! Sodium azide is highly toxic! Do not discharge to the plumbing system!). Dialyze (24" long cellulose acetate "Type C" membrane, Technicon, New York) an 8 mL aliquot against water pumped at 0.6 mL/min, pass the dialysate through column A to waste, after 10 min stop the dialysis and elute column A to waste with water, after 24 min backflush the contents of column A onto column B with mobile phase, after 5 min remove column A from the circuit, elute column B with mobile phase, monitor the effluent from column B. Meat. Blend 10 g homogenized meat with 20 mL saline, centrifuge, remove a 10 mL aliquot of the clear upper phase and add it to 1 mL 1% sodium azide (Caution! Sodium azide is highly toxic! Do not discharge to the plumbing system!). Dialyze (24" long cellulose acetate "Type C" membrane, Technicon, New York) an 8 mL aliquot against water pumped at 0.6 mL/min, pass the dialysate through column A to waste, after 10 min stop the dialysis and elute column A to waste with water, after 24 min backflush the contents of column A onto column B with mobile phase, after 5 min remove column A from the circuit, elute column B with mobile phase, monitor the effluent from column B. Eggs. Dilute 10 g homogenized whole egg with 10 mL saline, add 3 mL 10% sodium azide solution (Caution! Sodium azide is highly toxic! Do not discharge to the plumbing system!). Dialyze (24" long cellulose acetate "Type C" membrane, Technicon, New York) an 8 mL aliquot against water pumped at 0.6 mL/min, pass the dialysate through column A to waste, after 10 min stop the dialysis and elute column A to waste with water, after 24 min backflush the contents of column A onto column B with mobile phase, after 5 min remove column A from the circuit, elute column B with mobile phase, monitor the effluent from column B.

HPLC VARIABLES

Column: A 60 × 4 50-100 μm XAD-4 (Rohm & Haas); B 250 × 4.6 7 μm Cp TM-Spher C18 (Chrompack)

Mobile phase: MeCN:50 mM pH 6.85 sodium acetate buffer 12.5:87.5

Detector: UV 450 following post-column reaction. The column effluent mixed with 1.5% p-dimethylaminobenzaldehyde in 17% phosphoric acid and the mixture flowed through a 7.5 m × 0.5 mm ID knitted PTFE coil to the detector.

CHROMATOGRAM

Retention time: k' 3.5

Limit of detection: 5-10 ng/g

OTHER SUBSTANCES

Extracted: dapsone, sulfacetamide, sulfachlorpyrazine, sulfadiazine, sulfadimethoxine, sulfadoxine, sulfaguanidine, sulfamethazine, sulfamethoxazole, sulfanilamide, sulfaquinoxaline

Noninterfering: chloramphenicol, trimethoprim

Interfering: sulfamerazine, sulfatroxazole

KEY WORDS

post-column reaction; meat; column-switching; dialysis

REFERENCE

Aerts,M.M.L.; Beek,W.M.J.; Brinkman,U.A.T. Monitoring of veterinary drug residues by a combination of continuous flow techniques and column-switching high-performance liquid chromatography. I. Sulphonamides in egg, meat and milk using post-column derivatization with dimethylaminobenzaldehyde, *J.Chromatogr.*, **1988**, *435*, 97–112.

SAMPLE

Matrix: feed

Sample preparation: 20 g Ground feed + 100 mL solvent + 5 mL 8 μg/mL sulfamerazine in diluent, shake for 1 h, chill an aliquot in an ice bath for 2 h, centrifuge at 1650 g for 5 min, filter (0.2 μm), inject a 200 μL aliquot of the filtrate. (Prepare solvent by mixing 250 mL MeOH, 300 mL water, and 25 mL HCl, mix, add 15 mL diethylamine, mix, make up to 1 L with water. Prepare diluent by mixing 250 mL MeOH, 300 mL water, and 12.5 mL HCl, mix, add 15 mL diethylamine, mix, make up to 1 L with water.)

HPLC VARIABLES

Guard column: C8 or C18

Column: 250 × 4.67 μm Lichrosorb RP-18
Mobile phase: MeCN:2% acetic acid 17:83
Flow rate: 1-1.3
Injection volume: 200
Detector: UV 450 following post-column reaction. The column effluent mixed with the reagent pumped at 0.1-0.5 mL/min and the mixture flowed through a 3 m × 0.5 mm i.d. PTFE coil to the detector. (Prepare reagent by dissolving 1.5 g dimethylaminobenzaldehyde in 100 mL glacial acetic acid, add 60 mL MeOH, mix well, add 40 mL water, mix well, prepare fresh daily.)

CHROMATOGRAM
Retention time: 5.5
Internal standard: sulfamerazine (7)

OTHER SUBSTANCES
Extracted: sulfamethazine
Simultaneous: sulfadimethoxine, sulfaquinoxaline
Noninterfering: amino acids, amprolium, apramycin, arsanilic acid, bacitracin, hygromycin B, neomycin, nystatin, ormetoprim, procaine

KEY WORDS
post-column reaction

REFERENCE
Smallidge,R.L.; Kentzer,E.J.; Stringham,K.R.; Kim,E.H.; Lehe,C.; Stringham,R.W.; Mundell,E.C. Sulfamethazine and sulfathiazole determination at residue levels in swine feeds by reverse-phase liquid chromatography with post-column derivatization, *J.Assoc.Off.Anal.Chem.*, **1988**, *71*, 710–717.

SAMPLE
Matrix: feed, premix
Sample preparation: Shake premix or ground feed with 150 mM HCl in MeOH:water 25:75 for 1 h, dilute with 150 mM HCl in MeOH:water 25:75 to achieve a sulfonamide concentration of 5.5 μg/mL, filter (glass fiber), inject an aliquot.

HPLC VARIABLES
Guard column: 50 × 2 30-40 μm Perisorb RP-18
Column: 250 × 4.6 10 μm Partisil ODS-3
Mobile phase: MeCN:2% acetic acid 18:82
Flow rate: 1
Injection volume: 200
Detector: UV 450 following post-column reaction. The column effluent mixed with reagent pumped at 0.5 mL/min and the mixture flowed through a 3 m × 0.5 mm ID coil of PTFE tubing to the detector. (Prepare reagent by dissolving 3 g dimethylaminobenzaldehyde in 100 mL glacial acetic acid, add 60 mL MeOH, add 40 mL water, mix well.)

CHROMATOGRAM
Retention time: 8
Limit of quantitation: 1.65 μg/mL

KEY WORDS
post-column reaction

REFERENCE
Stringham,R.W.; Mundell,E.C.; Smallidge,R.L. Use of post-column derivatization in liquid chromatographic determination of sulfamethazine and sulfathiazole in feeds and feed premixes, *J.Assoc.Off.Anal.Chem.*, **1982**, *65*, 823–827.

SAMPLE
Matrix: formulations
Sample preparation: Powder tablets or pills. Weigh out an amount of powdered tablets or pills or capsule contents, dissolve in 5 mL EtOH, dilute with 150 mM HCl containing 40 mM sodium dodecyl sulfate. Dilute suspensions or drops with 150 mM HCl containing 40 mM sodium

dodecyl sulfate. Filter solutions if necessary. 10 mL Solution in 150 mM HCl containing 40 mM sodium dodecyl sulfate + 1 mL 100 mM sodium nitrite, let stand for 5 min, add 1 mL 300 mM sulfamic acid, let stand for 10 min, add 500 μL 30 mM N-(1-naphthyl)ethylenediamine dihydrochloride, make up to 25 mL with water, inject an aliquot.

HPLC VARIABLES
Guard column: 35 × 4.6 C18 (Scharlau)
Column: 125 × 4.6 5 μm Spherisorb ODS-2 C18
Mobile phase: Pentanol:50 mM sodium dodecyl sulfate 2.4:97.6, pH adjusted to 7 with 100 mM phosphate buffer
Flow rate: 1
Injection volume: 20
Detector: UV 490

CHROMATOGRAM
Retention time: 12
Limit of detection: 200 ng/mL

OTHER SUBSTANCES
Simultaneous: sulfacetamide, sulfadiazine, sulfaguanidine, sulfamerazine, sulfamethizole, sulfamethoxazole, sulfanilamide
Noninterfering: benzocaine

KEY WORDS
tablets; pills; capsules; suspensions; drops; derivatization

REFERENCE
Garcia-Alvarez-Coque,M.C.; Simo-Alfonso,E.F.; Ramis-Ramos,G.; Esteve-Romero,J.S. High-performance micellar liquid chromatography determination of sulphonamides in pharmaceuticals after azodye precolumn derivatization, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 237–245.

SAMPLE
Matrix: formulations, bulk
Sample preparation: Dilute with water to an idoxuridine concentration of 0.1%. Remove a 15 mL aliquot and add it to 2 mL sulfathiazole solution, make up to 20 mL with water, inject a 10 μL aliquot. (Prepare sulfathiazole solution by dissolving 120 mg sulfathiazole in 10 mL EtOH, make up to 100 mL with water.)

HPLC VARIABLES
Column: 300 × 4 10 μm μBondapak C18
Mobile phase: MeOH:water 13:87
Flow rate: 1.7
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 12.5
Internal standard: sulfathiazole

OTHER SUBSTANCES
Simultaneous: idoxuridine

KEY WORDS
eye drops; sulfathiazole is IS

REFERENCE
Carr,G.P.R. The development of British Pharmacopeia monographs for idoxuridine eye drops using high-pressure liquid chromatography for assay and for controlling related impurities, *J.Chromatogr.*, **1978**, *157*, 171–184.

SAMPLE

Matrix: milk

Sample preparation: Wash filter paper with 5 mL chloroform:acetone 2:1, discard filtrate. Extract 10 mL milk with 50 mL chloroform:acetone 2:1 by shaking for 4 min with periodic venting, let stand for 5 min, repeat extraction with 25 mL chloroform:acetone 2:1. Filter the organic layers, wash the filter paper with two 5 mL portions of chloroform:acetone 2:1. Evaporate the filtrate just to dryness under reduced pressure at 32 ± 2°, reconstitute the residue with 1 mL 13.6 g/L KH_2PO_4, vortex for 1 min, add 5 mL hexane, vortex for 1 min, let stand for 2 min, vortex for 1 min, let stand for at least 15 min, filter (2 μm) the aqueous layer, inject a 100 μL aliquot of the filtrate

HPLC VARIABLES

Guard column: 20 mm long Supelco guard column
Column: 250 × 4.6 LC-18-DB (Supelco)
Mobile phase: MeOH:13.6 g/L KH_2PO_4 12:88
Column temperature: 35
Flow rate: 1.5
Injection volume: 100
Detector: UV 265

CHROMATOGRAM

Retention time: 10.3
Limit of detection: 1.0 ppb
Limit of quantitation: 2.2 ppb

OTHER SUBSTANCES

Extracted: sulfadiazine, sulfamerazine, sulfamethazine, sulfamethizole, sulfanilamide, sulfapyridine

KEY WORDS

cow

REFERENCE

Dadgar,D.; Power,A. Applications of column-switching technique in biopharmaceutical analysis. I. High-performance liquid chromatographic determination of amitriptyline and its metabolites in human plasma, *J.Chromatogr.*, **1987**, *416*, 99–109.

SAMPLE

Matrix: milk

Sample preparation: 500 μL Milk + 2 g C18 material + 10 μL MeOH + 10 μL 12.5 μg/mL sulfamerazine in MeOH, let stand for 1 min, grind with a glass pestle until homogeneous, place in a 10 mL syringe barrel plugged with filter paper, place filter paper on top, compress to 4.5 mL with a plunger, restrict column outlet with a 100 μL pipette tip, wash with 8 mL hexane, remove excess hexane with positive pressure, elute with 8 mL dichloromethane, elute excess dichloromethane with positive pressure. Evaporate the eluate under a stream of nitrogen, dissolve the residue in 100 μL MeOH and 400 μL 17 mM orthophosphoric acid, sonicate for 5-10 min, centrifuge at 13600 g for 5 min, filter supernatant (0.45 μm), inject a 20 μL aliquot. (C18 material was Analytichem 40 μm 18% load endcapped. Add 22 g to a 50 mL syringe barrel wash with 2 column volumes of hexane, 2 volumes of dichloromethane, and 2 volumes of MeOH, vacuum aspirate until dry.)

HPLC VARIABLES

Column: 75 × 4 3 μm Supelcosil LC-18
Mobile phase: MeCN:17 mM orthophosphoric acid 10:90
Column temperature: 45
Flow rate: 1 for 5 min then 2 for remainder of run
Injection volume: 20
Detector: UV 270

CHROMATOGRAM

Retention time: 2
Internal standard: sulfamerazine (3)

Limit of detection: 62.5 ng/mL

OTHER SUBSTANCES
Simultaneous: sulfamethoxazole, sulfanilamide, sulfadiazine, sulfamethazine, sulfisoxazole, sulfadimethoxine

KEY WORDS
matrix solid-phase dispersion

REFERENCE
Long,A.R.; Short,C.R.; Barker,S.A. Method for the isolation and liquid chromatographic determination of eight sulfonamides in milk, *J.Chromatogr.*, **1990**, *502*, 87–94.

SAMPLE
Matrix: milk
Sample preparation: Wash filter paper with 5 mL chloroform:acetone 2:1, discard filtrate. Extract 10 mL milk with 50 mL chloroform:acetone 2:1 by shaking for 4 min with periodic venting, let stand for 5 min, repeat extraction with 25 mL chloroform:acetone 2:1. Filter the organic layers, wash the filter paper with two 5 mL portions of chloroform:acetone 2:1. Evaporate the filtrate just to dryness under reduced pressure at 32 ± 2°, reconstitute the residue with 1 mL 13.6 g/L KH_2PO_4, vortex for 1 min, add 5 mL hexane, vortex for 1 min, let stand for 2 min, vortex for 1 min, let stand for at least 15 min, filter (2 μm) the aqueous layer, inject a 100 μL aliquot of the filtrate

HPLC VARIABLES
Guard column: 20 mm long Supelco guard column
Column: 250 × 4.6 LC-18-DB (Supelco)
Mobile phase: MeOH:13.6 g/L KH_2PO_4 12:88
Column temperature: 35
Flow rate: 1.5
Injection volume: 100
Detector: UV 265

CHROMATOGRAM
Retention time: 10.3
Limit of detection: 1.0 ppb
Limit of quantitation: 2.2 ppb

OTHER SUBSTANCES
Extracted: sulfadiazine, sulfamerazine, sulfamethazine, sulfamethizole, sulfanilamide, sulfapyridine

KEY WORDS
cow

REFERENCE
Smedley,M.D.; Weber,J.D. Liquid chromatographic determination of multiple sulfonamide residues in bovine milk, *J.Assoc.Off.Anal.Chem.*, **1990**, *73*, 875–879.

SAMPLE
Matrix: milk
Sample preparation: 5 mL Milk + 100 μL concentrated HCl, sonicate for 15 s, centrifuge at 3000 g for 10 min, wash the precipitate with 2 mL water, centrifuge. Combine the aqueous layers and add 5 mL hexane, mix, centrifuge at 1500 g for 1 min, repeat the hexane wash. Evaporate the aqueous layer to dryness at low pressure, reconstitute with MeOH, centrifuge, evaporate the supernatant to dryness, reconstitute the residue with 3 mL water, inject a 50-500 μL aliquot on to column A and elute to waste with mobile phase A, after 3 min elute the contents of column A on to column B with mobile phase B and start the gradient, elute with mobile phase B and monitor the effluent from column B.

HPLC VARIABLES
Column: A 30 mm long 10 μm RP-18; B 150 × 4.6 5 μm Spherisorb ODS-2
Mobile phase: A 100 mM Ammonium acetate buffer or 1% formic acid (?); B Gradient. A was 100 mM ammonium acetate buffer or 1% formic acid (?). B was MeCN:water 70:30 containing

100 mM ammonium acetate or 1% formic acid (?). A:B from 100:0 to 80:20 over 0.5 min, maintain at 80:20,for 1 min, to 25:75 over 10 min.
Flow rate: 1
Injection volume: 50-500
Detector: UV 254 or MS, Finnigan TSQ 70 triple quadrupole, Finnigan TSP source and interface, interface 80-85°, source 250°, manifold 70°, collision gas argon 0.4 mTorr, collision energy 40-50 eV

CHROMATOGRAM
Retention time: 6.9
Limit of detection: 400 pg (LC-SIM), 5-20 ng (MS-scan), 2 ng (UV)

OTHER SUBSTANCES
Extracted: sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfamerazine, sulfamethazine, sulfamethizole, sulfanilamide, sulfapyridine, sulfaquinoxaline

KEY WORDS
cow; column-switching

REFERENCE
Abián,J.; Churchwell,M.I.; Korfmacher,W.A. High-performance liquid chromatography-thermospray mass spectrometry of ten sulfonamide antibiotics. Analysis in milk at the ppb level, *J.Chromatogr.*, **1993**, *629*, 267–276.

SAMPLE
Matrix: milk, urine
Sample preparation: Urine. Filter (Rainin glassfiber microfilter and Rainin 0.45 μm nylon-66 filter), inject an aliquot. Milk. Filter (Rainin glassfiber microfilter), inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm YMC-Pack ODS-AQ (YMC)
Mobile phase: MeOH:buffer 6:94 pH adjusted to 3.0 (Buffer was 70 mM in sodium dodecyl sulfate and 20 mM in NaH_2PO_4.)
Column temperature: 40
Detector: UV 254

CHROMATOGRAM
Retention time: 5.17

OTHER SUBSTANCES
Extracted: sulfacetamide, sulfabenzamide, sulfadiazine, sulfamerazine, sulfadimethoxine, sulfamethazine, sulfamethoxypyridazine, sulfamonomethoxine, sulfaquinoxaline, sulfisomidine

KEY WORDS
human; cow; micellar liquid chromatography

REFERENCE
Yang,S.; Khaledi,M.G. Micellar liquid chromatographic separation of sulfonamides in physiological samples using direct on-column injection, *J.Chromatogr.A*, **1995**, *692*, 311–318.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 40 × 4.6 25-37 μm Co:Pell ODS
Column: 250 × 4.6 10 μm Partisil PXS ODS-2
Mobile phase: MeCN:MeOH 10:90
Flow rate: 0.7
Injection volume: 20

Detector: UV 254

CHROMATOGRAM
Retention time: 3.9

OTHER SUBSTANCES
Simultaneous: oxybenzone, padimate-O, propyl paraben

REFERENCE
Tan,H.S.I.; Sih,R.; Moseley,S.E.; Lichtin,J.L. Assay of mixtures of padimate-O and oxybenzone in sunscreen formulations by high-performance liquid chromatography, *J.Chromatogr.*, **1984**, *291*, 275–282.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 20:1.5:0.5:78
Flow rate: 1.5
Injection volume: 10
Detector: UV

CHROMATOGRAM
Retention time: k' 1.71

REFERENCE
Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4 OmniPac PCX-500 (Dionex)
Mobile phase: Gradient. A was MeCN:110 mM perchloric acid and 20 mM sodium acetate 27:73. B was MeCN:110 mM perchloric acid and 100 mM sodium acetate 50:50. A:B from 100:0 to 0:100 over 5 min, then re-equilibrate.
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 3.2

OTHER SUBSTANCES
Simultaneous: sulfamerazine, sulfamethazine, sulfamethizole, sulfamethoxazole, sulfanilamide, sulfanilic acid, sulfisoxazole

REFERENCE
Slingsby,R.W.; Rey,M. Determination of pharmaceuticals by multi-phase chromatography: Combined reversed phase and ion exchange in one column, *J.Liq.Chromatogr.*, **1990**, *13*, 107–134.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in MeOH:water 25:75, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 250 × 2.1 5 μm 201TP (Vydac)

Mobile phase: Gradient. A was MeCN containing 0.1% trifluoroacetic acid. B was water containing 0.1% trifluoroacetic acid. A:B from 5:95 to 40:60 over 20 min.
Flow rate: 0.2
Injection volume: 5
Detector: UV 270 or MS, Sciex API III triple quadrupole, IonSpray interface

CHROMATOGRAM
Retention time: 7.97

OTHER SUBSTANCES
Simultaneous: phthalylsulfathiazole, succinylsulfathiazole, sulfabenzamide, sulfacetamide, sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfaguanidine, sulfamerazine, sulfameter, sulfamethazine, sulfamethizole, sulfamethoxazole, sulfamethoxypyridazine, sulfamoxole, sulfanilamide, sulfapyridine, sulfaquinoxaline, sulfisomidine, sulfisoxazole

REFERENCE
Pleasance,S.; Blay,P.; Quilliam,M.A.; O'Hara,G. Determination of sulfonamides by liquid chromatography, ultraviolet diode array detection and ion-spray tandem mass spectrometry with application to cultured salmon flesh, *J.Chromatogr.*, **1991**, *558*, 155–173.

SAMPLE
Matrix: solutions
Sample preparation: Inject an aliquot of a solution in MeOH.

HPLC VARIABLES
Column: 100 × 4.6 3 μm Microsphere C18 (Chrompack)
Mobile phase: MeCN:MeOH:THF:buffer 0.1:14:0.5:85.4 (Prepare buffer by dissolving 6.80 g KH_2PO_4 in 1 L water, adjust pH to 3.0 with concentrated phosphoric acid, add 4.15 mL triethylamine, add 10 mL glacial acetic acid.)
Flow rate: 1
Injection volume: 20
Detector: UV 260

CHROMATOGRAM
Retention time: k' 2.10

OTHER SUBSTANCES
Simultaneous: sulfachloropyridazine, sulfamerazine, sulfamethoxypyridazine, sulfapyridine, sulfisomidine

REFERENCE
Wieling,J.; Coenegracht,P.M.J.; Doornbos,D.A.; Jonkman,J.H.G. Robustness testing of an optimized reversed-phase high-performance liquid chromatographic system for the separation of six sulphonamides using the rules of error propagation, *J.Chromatogr.*, **1993**, *635*, 195–202.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Guard column: Sentry (Waters)
Column: 150 × 4.6 Symmetry C8 (Waters)
Mobile phase: MeOH:water:glacial acetic acid 20:79:1
Column temperature: 25
Flow rate: 1
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 3.7

OTHER SUBSTANCES
Simultaneous: sulfanilamide, sulfadiazine, sulfamerazine, sulfamethazine, succinylsulfathiazole

REFERENCE
Capparella,M.; Foster,W.,III; Larrousse,M.; Phillips,D.J.; Pomfret,A.; Tuvim,Y. Characteristics and applications of a new high-performance liquid chromatography guard column, *J.Chromatogr.A*, **1995**, *691*, 141–150.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 0.35 5 μm Vydac IDI-TP C18 TMS capped
Mobile phase: Gradient. MeCN:MeOH:buffer 0:0:100 at start of run, to 0:5:95 after injection (step gradient), to 0:8:92 over 7 min, to 6:0:94 (step gradient), maintain at 6:0:94 for 14 min, to 0:16:84 over 5 min, to 0:18:82 over 5 min, to 0:30:70 over 5 min. (Buffer was 1 mM pH 2.72 phosphate buffer.)
Column temperature: 30
Flow rate: 0.006
Injection volume: 1
Detector: UV 270

CHROMATOGRAM
Retention time: 24

OTHER SUBSTANCES
Simultaneous: diaveridine, phthalyl sulfathiazole, pyrimethamine, succinyl sulfathiazole, sulfabenzamide, sulfacetamide, sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfaguanidine, sulfamerazine, sulfameter, sulfamethazine, sulfamethizole, sulfamethoxazole, sulfamethoxypyridazine, sulfamoxole, sulfanilamide, sulfanilic acid, sulfaquinoxaline, sulfisomidine, sulfisoxazole, trimethoprim
Interfering: sulfapyridine

KEY WORDS
capillary HPLC

REFERENCE
Ricci,M.C.; Cross,R.F. High performance liquid chromatographic analyses of sulphonamides and dihydrofolate reductase inhibitors. II. Separations in acetonitrile modified solutions, ternary gradient studies & flow programming, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 547–564.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 0.35 5 μm Vydac IDI-TP C18 TMS capped
Mobile phase: Gradient. MeOH:buffer 0:100 at start of run, to 10:90 after injection (step gradient), to 12:88 over 30 min, to 18:82 over 5 min, to 30:70 over 5 min. (Buffer was 1 mM pH 2.72 phosphate buffer
Column temperature: 30
Flow rate: 0.006
Injection volume: 1
Detector: UV 270

CHROMATOGRAM
Retention time: 21

OTHER SUBSTANCES
Simultaneous: diaveridine, phthalyl sulfathiazole, pyrimethamine, succinyl sulfathiazole, sulfabenzamide, sulfacetamide, sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfaguanidine, sulfamerazine, sulfameter, sulfamethazine, sulfamethizole, sulfamethoxazole, sulfamethoxypyridazine, sulfamoxole, sulfanilamide, sulfanilic acid, sulfaquinoxaline, sulfisomidine, sulfisoxazole, trimethoprim

Interfering: sulfapyridine

KEY WORDS
capillary HPLC

REFERENCE
Ricci,M.C.; Cross,R.F. High-performance liquid chromatographic analyses of sulphonamides and dihydrofolate reductase inhibitors. I. Separations in methanol-modified solutions, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 365–381.

SAMPLE
Matrix: tissue
Sample preparation: Condition a 500 mg Chromabond SA cation-exchange SPE cartridge (Macherey-Nagel) with 6 mL hexane, dry under vacuum for 10 min, condition with 6 mL dichloromethane:acetone:acetic acid 50:50:2, do not allot to go dry. Homogenize (Polytron) 10 g sample with 60 mL dichloromethane:acetone 50:50 for 30 s, rinse the apparatus with 2-3 mL dichloromethane:acetone 50:50, centrifuge the mixture at 2500 rpm for 10 min. Filter (cotton wool) the supernatant and wash it through with a little dichloromethane:acetone 50:50, add 5 mL acetic acid to the filtrate, mix, remove one tenth of this mixture and add it to the SPE cartridge at 2 mL/min, do not allow the SPE cartridge to run dry, wash with 5 mL water, wash with 5 mL MeOH, dry under vacuum for 10 min, pass gaseous ammonia through the SPE cartridge until the acid is neutralized (when air is passed through the cartridge moist pH paper should turn blue), elute with 3 mL MeOH at 1-2 mL/min, carefully evaporate to dryness under reduced pressure (100 mbar) at 40°, reconstitute with 500 µL initial mobile phase, centrifuge, inject a 50 µL aliquot of the supernatant.

HPLC VARIABLES
Column: 125 × 4 5 µm LiChrospher 100 RP-18
Mobile phase: Gradient. A was MeCN:20 mM pH 5 sodium acetate buffer 5.5:94.5. B was MeCN:EtOH:20 mM pH 5 sodium acetate buffer 50:10:40. A:B from 100:0 to 0:100 over 32 min (concave gradient), return to initial conditions over 4 min, re-equilibrate at initial conditions for 10 min.
Flow rate: 0.8
Injection volume: 50
Detector: UV 270, F ex 395 em 495 following post-column reaction. The column effluent mixed with ice-cold reagent pumped at 0.3 mL/min and this mixture flowed through a 2.3 m × 0.5 mm ID coil in a cooled ultrasonic bath to the detector. (Prepare reagent by dissolving 25 mg fluorescamine in 25 mL MeCN and adding 75 mL buffer and 200 µL mercaptoethanol. Buffer was 20 mM NaH_2PO_4 adjusted to pH 3 with 1 M phosphoric acid.)

CHROMATOGRAM
Retention time: 15
Limit of detection: 0.5-5 ppb

OTHER SUBSTANCES
Extracted: sulfachlorpyridazine, sulfadiazine, sulfadimethoxine, sulfadoxine, sulfaguanidine, sulfamerazine, sulfamethazine (sulfadimidine), sulfamethizole, sulfamethoxypyridazine, sulfanilamide, sulfapyridine

KEY WORDS
post-column reaction; muscle; kidney; SPE

REFERENCE
Pacciarelli,B.; Reber,S.; Douglas,C.; Dietrich,S.; Etter,R. Determination of 12 sulfonamides in meat and kidney by HPLC with post-column derivatization, *Mitt.geb.Lebensmittelunters.Hyg.*, **1991**, *82*, 45–55.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Polytron) 10 g ground tissue with 40 mL acetone, centrifuge at 2800 g for 5 min, filter (paper) the supernatant. Homogenize (Polytron) the residue with 20 mL acetone for 1 min, centrifuge, filter. Combine the filtrates and add 60 mL 125 mM HCl, wash twice with 50 mL portions of n-hexane, add 10 mL 1 M pH 5.2 acetate buffer, adjust pH

to 5.0-5.1 with 5 M NaOH, extract with 60 ml and 40 mL portions of ethyl acetate, combine the organic layers, evaporate to about 2 mL under reduced pressure at 45°C, add about 15 mL EtOH, evaporate to dryness under reduced pressure at 50°, reconstitute immediately with 5-7 mL dichloromethane. Add to an 85 mm long column of silica gel made up in dichloromethane, rinse the flask twice with 1-2 mL portions of dichloromethane, add the rinses to the column, elute with 40 mL acetone:dichloromethane (60:40), elute to waste until the acetone front (visible against a dark background) is about 10 mm from the end of the column, collect the remaining eluate (Mitt. Gebiete. Lebensm. Hyg. 1990, 81, 522). Add 150 μL 10 μg/mL sulfabenzamide to the eluate, evaporate to dryness under reduced pressure at 45°, reconstitute the residue in 300 μL MeOH:water 50:50, filter (0.45 μm), inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 4 × 4 LiChrospher 5 μm 100 RP-18
Column: 250 × 4 5 μm Spherisorb ODS2
Mobile phase: MeCN:buffer 20:80 (Prepare buffer by dissolving 3.85 g ammonium acetate in 950 mL water, ad just pH to 4.00 with acetic acid, make up to 1 L with water.)
Column temperature: 35
Flow rate: 1
Injection volume: 20
Detector: UV 550 following post-column reaction. The column effluent mixed with ice-cold reagent pumped at 0.2 mL/min and the mixture flowed through a 25 cm × 0.33 mm ID coil. The effluent from this coil mixed with ice-cold 20 mg/mL ammonium sulfamate in water pumped at 0.2 mL/min and this mixture flowed through an ice-cooled 200 cm × 0.33 mm ID coil. The effluent from this coil mixed with ice-cold 4 mg/mL N-(1-naphthyl)ethylenediamine hydrochloride in water pumped at 0.2 mL/min and this mixture flowed through a 60 cm × 0.33 mm ID coil to the detector. (Reagent was 800 mg sodium nitrite dissolved in 150 mL water and 50 mL concentrated HCl.)

CHROMATOGRAM

Retention time: 4.5
Internal standard: sulfabenzamide (8.8)
Limit of detection: 2 ppb

OTHER SUBSTANCES

Extracted: sulfacetamide, sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfamerazine, sulfamethazine, sulfamethoxazole, sulfamethoxypyridazine, sulfanilamide
Interfering: sulfapyridine

KEY WORDS

post-column reaction; muscle; liver; kidney; SPE

REFERENCE

Guggisberg,D.; Mooser,A.E.; Koch,H. Screening method for the quantitative determination of twelve sulfonamides in meat, liver, and kidney by HPLC with online post-column derivatization, *Mitt.geb. Lebensmittelunters.Hyg.*, **1993**, *84*, 263–273.

SAMPLE

Matrix: tissue
Sample preparation: Condition a 3 mL 500 mg Sep-Pak SPE cartridge with 20 mL MeOH and 20 mL water. 5 g Homogenized tissue + 40 μL 20 μg/mL sulfaethoxypyridazine in water + 25 mL chloroform, shake mechanically for 2 min, centrifuge at 3000 g for 5 min, remove the supernatant and separate the layers. Add the aqueous layer to the residue and repeat the extraction. Combine the chloroform layers and add 10 mL 10% NaCl in 100 mM NaOH, shake vigorously for 1 min, remove the upper aqueous layer and centrifuge it at 1500 g for 10 min. Remove 8 mL of the upper aqueous layer and add it to 10 mL 1 M pH 6 NaH_2PO_4, vortex for 20 s, add to the SPE cartridge, wash with 20 mL water, elute with 1 mL MeCN. Evaporate the eluate to dryness under a stream of nitrogen at 50°, reconstitute in 2 mL mobile phase, vortex for 20 s, heat at 50° for 5 min, cool, filter (Gelman Acrodisc 0.45 μm), inject a 20-50 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Spherisorb C18 ODS
Mobile phase: MeCN:10 mM pH 4.6 ammonium acetate 28:72

Flow rate: 1.2
Injection volume: 20-50
Detector: UV 265 or MS, VG TRIO 2 quadrupole, ion source 189°, capillary jet 320

CHROMATOGRAM
Retention time: 4.9
Internal standard: sulfaethoxypyridazine (12.8)

OTHER SUBSTANCES
Extracted: sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfadoxine, sulfamerazine, sulfamethazine, sulfamethoxazole, sulfamethoxypyridazine

KEY WORDS
cow; pig; muscle; liver; SPE

REFERENCE
Boison,J.O.; Keng,L.J.-Y. Determination of sulfadimethoxine and sulfamethazine residues in animal tissues by liquid chromatography and thermospray mass spectrometry, *J.AOAC Int.*, **1995**, *78*, 651–658.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Ultra-Turrax T-25 with S25N dispersing tool) 10 g chopped fish and 10 mL mobile phase A at high speed for 30 s, add 90 mL MeCN, shake at low speed on shaker, centrifuge at 1500 rpm for 10 min, remove the supernatant, add 30 mL MeCN to the solid, shake, centrifuge, decant the supernatant. Combine the supernatants, add 100 mL water, add 2 mL diethylene glycol, add 60 mL dichloromethane, shake for 3 min, remove the organic layer, repeat the extraction with 40 mL dichloromethane. Combine the organic layers and evaporate in a rotary evaporator at 65° to ca. 2 mL, wash into a smaller tube with two 2 mL portions of MeOH, concentrate to about 1 mL with a stream of nitrogen at 65°, dilute to 4.5 mL with 200 mM phosphoric acid, add 5 mL hexane, vortex, centrifuge for 15 min, discard upper hexane layer. Dilute the lower aqueous layer to 5 mL with 200 mM phosphoric acid, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: C18
Column: 150 × 4.6 3.5 μm Symmetry C18 (Waters)
Mobile phase: Gradient. A was MeCN:MeOH:2% acetic acid in water 5:10:85. B was MeCN:MeOH:2% acetic acid in water 25:10:65. A:B from 100:0 to 0:100 over 25 min, maintain at 0:100 for 5 min.
Flow rate: 1
Injection volume: 20
Detector: F ex 400 em 495 following post-column reaction. The column effluent mixed with 500 μg/mL fluorescamine in MeCN:MeOH:2% acetic acid 52.5:5:42.5 pumped at 0.2 mL/min and the mixture flowed through a 10.7 m × 0.4 mm i.d. coil of PTFE tubing at 70° to the detector.

CHROMATOGRAM
Retention time: 6.5
Limit of quantitation: 1 ng/g

OTHER SUBSTANCES
Extracted: sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfadoxine, sulfamerazine, sulfamethazine, sulfamethizole, sulfamethoxazole, sulfamethoxypyridazine, sulfamonomethoxine, sulfanilamide, sulfapyridine, sulfaquinoxaline

KEY WORDS
fish; salmon; post-column reaction

REFERENCE
Gehring,T.A.; Rushing,L.G.; Thompson,H.C.,Jr. Determination of sulfonamides in edible salmon tissue by liquid chromatography with postcolumn derivatization and fluorescence detection, *J.AOAC Int.*, **1997**, *80*, 751–755.

SAMPLE
Matrix: urine
Sample preparation: 2 mL Urine + 10 mL 150 mM HCl containing 40 mM sodium dodecyl sulfate + 1 mL 100 mM sodium nitrite, let stand for 5 min, add 1 mL 300 mM sulfamic acid, let stand for 10 min, add 500 μL 30 mM N-(1-naphthyl)ethylenediamine dihydrochloride, make up to 25 mL with water, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 35 × 4.6 C18 (Scharlau)
Column: 125 × 4.6 5 μm Spherisorb ODS-2 C18
Mobile phase: Pentanol:50 mM sodium dodecyl sulfate 2.4:97.6, pH adjusted to 7 with 100 mM phosphate buffer
Flow rate: 1
Injection volume: 20
Detector: UV 490

CHROMATOGRAM
Retention time: 10.5
Limit of detection: 200 ng/mL

OTHER SUBSTANCES
Extracted: sulfadiazine, sulfaguanidine, sulfathiazole, sulfamethoxazole

KEY WORDS
derivatization

REFERENCE
Simó-Alfonso,E.F.; Ramis-Ramos,G.; García-Alvarez-Coque,M.C.; Esteve-Romero,J.S. Determination of sulphonamides in human urine by azo dye precolumn derivatization and micellar liquid chromatography, *J.Chromatogr.B*, **1995**, *670*, 183–187.

SAMPLE
Matrix: water
Sample preparation: Adjust 50 mL wastewater to pH 6.6 with acetic acid, add 5 mL 1 mg/mL niacin in 0.5 mM HCl, add 50 mL ethyl acetate, shake vigorously for 5 min, let stand for 1 min, transfer the ethyl acetate layer to a flask, extract the residual aqueous layer with two 20 mL portions of ethyl acetate. Combine the organic layers and evaporate them at 90° to about 500 μL, dissolve the residue in 5 mL 10 mM HCl, make up to 50 mL with water, inject an aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Inertsil ODS-2 (Vercopak)
Mobile phase: MeOH:buffer 20:80 (Buffer was 100 mM sodium acetate adjusted to pH 6.6 with 10 mM acetic acid.)
Flow rate: 1
Injection volume: 20
Detector: UV 260

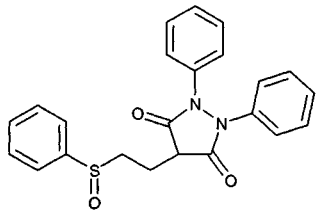
CHROMATOGRAM
Retention time: 5.5
Internal standard: niacin (3.3)

OTHER SUBSTANCES
Extracted: sulfamethazine, sulfacetamide, sulfamethoxazole, sulfadiazine, sulfamerazine, sulfamonomethoxine

KEY WORDS
wastewater

REFERENCE
Jen,J.-F.; Lee,H.-L.; Lee,B.-N. Simultaneous determination of seven sulfonamide residues in swine wastewater by high-performance liquid chromatography, *J.Chromatogr.A*, **1998**, *793*, 378–382.

Sulfinpyrazone



Molecular formula: $C_{23}H_{20}N_2O_3S$
Molecular weight: 404.49
CAS Registry No.: 57-96-5
Merck Index: 9121
Lednicer No.: 1 238

SAMPLE
Matrix: bile, blood
Sample preparation: Plasma, urine, or bile + 50 μL 100 μg/mL fenbufen in MeOH + 2 mL 1 M HCl + 5 mL chlorobutane:1,2-dichloroethane 80:20, extract, centrifuge. Remove the organic layer and add it to 400 μL 100 mM NaOH, shake for 5 min, centrifuge, inject a 200 μL aliquot of the aqueous layer.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Ultrasphere ODS
Mobile phase: MeOH:100 mM pH 5.6 phosphate buffer 50:50
Flow rate: 1.6
Injection volume: 200
Detector: UV 254

CHROMATOGRAM
Internal standard: fenbufen

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; urine; rabbit; pharmacokinetics

REFERENCE
Strong,H.A.; Renwick,A.G.; George,C.F. The site of reduction of sulphinpyrazone in the rabbit, *Xenobiotica*, **1984**, *14*, 815–826.

SAMPLE
Matrix: blood
Sample preparation: Condition a Sep-pak C18 SPE cartridge with 3 mL MeOH and 3 mL 10 mM tetrabutylammonium phosphate. 1 mL Plasma + 1 mL 20 mM tetrabutylammonium phosphate in MeOH:water 50:50, agitate for 30 s, add to the SPE cartridge, wash with 1 mL 10 mM tetrabutylammonium phosphate MeCN:MeOH:water 15:15:70, elute with 1 mL MeOH, inject a 20 μL aliquot of the eluate.

HPLC VARIABLES
Column: 300 × 4 Bondapak C18
Mobile phase: MeOH:water 56:44 containing 5 mM tetrabutylammonium phosphate (PIC A)
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 5
Limit of quantitation: 200 ng/mL

KEY WORDS
plasma; SPE

REFERENCE
Patel,N.J.; Kildsig,D.O.; Banker,G.S.; Mayer,P.R.; Gonzalez,M.A. Paired-ion high-performance liquid chromatographic assay for sulfinpyrazone in plasma, *J.Pharm.Sci.*, **1982**, *71*, 1413–1415.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 200 μL 42 μg/mL naproxen + 500 μL MeCN, shake gently, centrifuge at 10000 g for 2 min. Remove the supernatant and dilute with an equal volume of water, inject a 50-200 μL aliquot.

HPLC VARIABLES
Guard column: Bondapak C18/Corasil
Column: 115 × 8 5 μm Radial-Pak C18 (Waters)
Mobile phase: MeCN:20 mM pH 7.0 phosphate buffer 26:74
Flow rate: 2
Injection volume: 50-200
Detector: UV 254

CHROMATOGRAM
Retention time: 7
Internal standard: naproxen (4.5)
Limit of detection: 100 ng/mL

OTHER SUBSTANCES
Extracted: metabolites
Simultaneous: ibuprofen
Noninterfering: lidocaine, metoprolol, propranolol

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Tam,Y.K.; Ferguson,S.M.; Yau,M.L.; Wyse,D.G. Simple and rapid high-performance liquid chromatographic method for the analysis of sulfinpyrazone and four of its metabolites in human plasma, *J.Chromatogr.*, **1984**, *310*, 438–444.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 1 mL Plasma + 10 μg naproxen + 1 mL 1 M HCl + 500 μL 20 mg/mL sodium sulfite + 4 mL dichloromethane:1-chlorobutane 75:25, shake for 15 min, centrifuge at 2000 g for 10 min. Remove 3 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 100 μL 2% sodium sulfite, vortex, inject a 10 μL aliquot. Urine. 500 μL Urine + 500 μL water + 25 μg naproxen + 1 mL 1 M HCl + 500 μL 20 mg/mL sodium sulfite + 4 mL dichloromethane:1-chlorobutane 75:25, shake for 15 min, centrifuge at 2000 g for 10 min. Remove 3 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 100 μL 2% sodium sulfite, vortex, inject a 10 μL aliquot.

HPLC VARIABLES
Guard column: 25 × 4 35-50 μm Bondapak C18 Corasil
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: Gradient. A was MeCN:100 mM ammonium acetate 22:78. B was MeCN. A:B from 0:100 to 100:0 over 15 min (Waters convex curve 7), re-equilibrate for 5 min.
Flow rate: 2
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 5.86
Internal standard: naproxen (9.85)
Limit of detection: 500 ng/mL (urine), 100 ng/mL (plasma)

OTHER SUBSTANCES
Extracted: metabolites
Simultaneous: antipyrine, phenprocoumon
Noninterfering: ampicillin, aspirin, azlocillin, cimetidine, cotinine, digoxin, heparin, hippuric acid, lidocaine, methaqualone, metoprolol, mezlocillin, neostigmine, nicotine, pindolol, procainamide, propranolol, quinidine, salicylamide, salicylic acid, secobarbital, theobromine, theophylline, uric acid, ascorbic acid, vitamin B, warfarin

KEY WORDS
plasma; pharmacokinetics

REFERENCE
de Vries,J.X.; Staiger,C.; Wang,N.S.; Schlicht,F. Analysis of sulfinpyrazone and its metabolites in human plasma and urine by high-performance liquid chromatography, *J.Chromatogr.*, **1983**, *277*, 408–413.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. Evaporate 50-100 μL 100 μg/mL butyl 4-hydroxybenzoate in MeOH into the bottom of a glass tube using a stream of air at 30°, add 500 μL plasma, add 500 μL 600 mM HCl, add 6 mL 1-chlorobutane:chloroform 50:50, shake mechanically for 20 min, centrifuge at 1400 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of air at 30°, reconstitute the residue in 1 mL EtOH:water 50:50, inject a 10-20 μL aliquot. Urine. Evaporate 50-100 μL 100 μg/mL butyl 4-hydroxybenzoate in MeOH into the bottom of a glass tube using a stream of air at 30°, add 500 μL urine, add 500 μL 600 mM HCl, add 6 mL 1-chlorobutane:chloroform 50:50, shake mechanically for 20 min, centrifuge at 1400 g for 10 min. Remove the organic layer and add it to 500 μL 500 mM pH 4.5 citrate buffer, shake for 5 min, centrifuge at 1400 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of air at 30°, reconstitute the residue in 1 mL EtOH:water 50:50, inject a 10-20 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Lichrosorb RP-8
Mobile phase: EtOH:10 mM pH 2.5 citrate buffer 48:52
Column temperature: 40
Flow rate: 1
Injection volume: 10-20
Detector: UV 254

CHROMATOGRAM
Retention time: 4
Internal standard: butyl 4-hydroxybenzoate (6)
Limit of quantitation: 100 ng/mL (urine), 25 ng/mL (plasma)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Lentjes,E.G.W.M.; Tan,Y.; van Ginneken,C.A.M. Determination of sulfinpyrazone and four metabolites in plasma and urine by high pressure liquid chromatography, *Pharm.Weekbl.[Sci.]*, **1985**, *7*, 252–259.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 50 μg/mL solution in the mobile phase, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 7 μm Lichrosorb RP 18
Mobile phase: MeOH:water 50:50 containing 1% acetic acid
Flow rate: 1

Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 8.94

OTHER SUBSTANCES
Simultaneous: kebuzone, oxyphenbutazone, phenylbutazone

REFERENCE
Nivaud-Guernet,E.; Guernet,M.; Ivanovic,D.; Medenica,M. Effect of eluent pH on the ionic and molecular forms of the non-steroidal anti-inflammatory agents in reversed-phase high-performance liquid chromatography, *J.Liq.Chromatogr.*, **1994**, *17*, 2343–2357.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

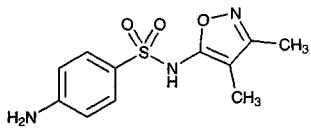
CHROMATOGRAM
Retention time: 5.71 (A), 5.33 (B)

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

Sulfisoxazole



Molecular formula: $C_{12}H_{13}N_3O_3S$
Molecular weight: 267.31
CAS Registry No.: 127-69-5, 4299-60-9 (diolamine)
Merck Index: 9125
Lednicer No.: 1 124

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 200 μL Plasma + 400 μL 0.2 μg/mL N^4-acetylsulfamethoxazole in MeOH, vortex for 10 s, centrifuge at 2000 rpm for 10 min. Remove the supernatant and evaporate it to 100 μL under a stream of nitrogen, inject a 50 μL aliquot. Urine. 100 μL Urine + 200 μL 12 μg/mL N^4-acetylsulfamethoxazole in MeOH, vortex for 10 s, centrifuge at 2000 rpm for 10 min, inject a 10 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 250 × 4.6 10 μm Lichrosorb RP-18 Hibar II
Mobile phase: MeOH:water:glacial acetic acid 32:68:0.06, pH adjusted to 4.7 with 4 M NaOH
Flow rate: 1.2
Injection volume: 10-50
Detector: UV 254

CHROMATOGRAM
Retention time: 6.5
Internal standard: N^4-acetylsulfamethoxazole (11.5)
Limit of quantitation: 50 ng/mL

OTHER SUBSTANCES
Extracted: acetylsulfisoxazole

KEY WORDS
plasma

REFERENCE
Jung,D.; Oie,S. "High-pressure" liquid chromatography of sulfisoxazole and N^4-acetylsulfisoxazole in body fluids, *Clin.Chem.*, **1980**, *26*, 51–54.

SAMPLE
Matrix: formulations
Sample preparation: Tablets. Weigh out ground tablets to contain 500 mg sulfisoxazole, add 25 mL MeOH, shake mechanically for 30 min, make up to 100 mL with MeOH, filter. Remove a 5 mL aliquot of the filtrate and add it to 15 mL 1 mg/mL sulfabenzamide in MeOH, mix, make up to 100 mL with MeOH, inject an aliquot. Injections, ophthalmic solutions. Measure out amount containing 200 mg sulfisoxazole, make up to 200 mL with MeOH. Remove a 25 mL aliquot and add it to 25 mL 1 mg/mL sulfabenzamide in MeOH, mix, make up to 100 mL with MeOH, inject an aliquot. Ointments. Weigh out ointment containing 50 mg sulfisoxazole, add 25 mL MeOH:water 50:50, wash twice with 50 mL portions of n-heptane. Extract the n-heptane layers three times with 25 mL portions of MeOH:water 50:50. Combine all the MeOH:water layers and add 50 mL 1 mg/mL sulfabenzamide in MeOH, mix, make up to 200 mL with MeOH, inject an aliquot.

HPLC VARIABLES
Column: 300 × 3.9 μBondapak C18

Mobile phase: MeCN:water:acetic acid 22.5:76.5:1
Flow rate: 1.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 7
Internal standard: sulfabenzamide (8)

OTHER SUBSTANCES
Simultaneous: sulfacetamide, sulfachlorpyridazine, sulfadiazine, sulfadimethoxine, sulfamerazine, sulfamethazine, sulfamethizole, sulfamethoxazole, sulfanilamide, sulfapyridine

KEY WORDS
tablets; injections; ophthalmic solutions; ointments

REFERENCE
Roos,R.W. High pressure liquid chromatographic determination of sulfisoxazole in pharmaceuticals and separation patterns of other sulfonamides, *J.Assoc.Off.Anal.Chem.*, **1981**, *64*, 851–854.

SAMPLE
Matrix: formulations
Sample preparation: Extract 1 mL suspension with three 15 mL aliquots of chloroform, combine the organic layers and make up to 50 mL with chloroform, filter (0.45 μm silver membrane, Selas Corp.). Evaporate a 2 mL aliquot of the filtrate to dryness under a stream of nitrogen, reconstitute with 5 mL 330 μg/mL benzanilide in MeCN, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 300 × 4 10 μm μBondapak C18
Mobile phase: MeCN:water 40:60
Flow rate: 1.5
Injection volume: 5
Detector: UV 254

CHROMATOGRAM
Retention time: 3
Internal standard: benzanilide (11)

OTHER SUBSTANCES
Simultaneous: acetylsulfisoxazole, sulfanilamide, sulfanilic acid
Noninterfering: erythromycin ethylsuccinate

KEY WORDS
oral suspensions; suspensions

REFERENCE
Elrod,L.,Jr.; Cox,R.D.; Plasz,A.C. Analysis of oral suspensions containing sulfonamides in combination with erythromycin ethylsuccinate, *J.Pharm.Sci.*, **1982**, *71*, 161–166.

SAMPLE
Matrix: formulations
Sample preparation: Tablets. Weigh out ground tablets to contain 500 mg sulfisoxazole, add 25 mL MeOH, shake mechanically for 30 min, make up to 100 mL with MeOH, filter. Remove a 20 mL aliquot of the filtrate and make up to 100 mL with MeOH. Remove a 5 mL aliquot and add it to 5 mL 800 μg/mL sulfadimethoxine in MeOH, mix, make up to 100 mL with mobile phase, inject an aliquot. Injections, ophthalmic solutions. Measure out amount containing 200 mg sulfisoxazole, make up to 200 mL with MeOH. Remove a 5 mL aliquot and add it to 5 mL 800 μg/mL sulfadimethoxine in MeOH, mix, make up to 100 mL with mobile phase, inject an aliquot. Ointments. Weigh out ointment containing 50 mg sulfisoxazole, add 50 mL n-heptane, shake to disperse, extract three times with 25 mL portions of MeOH:water 2:1, wash each extract with 50 mL n-heptane. Combine all the MeOH:water layers and make up to 100 mL

with MeOH. Remove a 10 mL aliquot and add it to 5 mL 800 μg/mL sulfadimethoxine in MeOH, mix, make up to 100 mL with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:water:acetic acid 30:69:1
Flow rate: 2
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 4
Internal standard: sulfadimethoxine (5.5)

KEY WORDS
tablets; injections; ophthalmic solutions; ointments

REFERENCE
Roos,R.W. High pressure liquid chromatographic determination of sulfisoxazole in dosage forms: collaborative study, *J.Assoc.Off.Anal.Chem.*, **1983**, *66*, 1182–1185.

SAMPLE
Matrix: milk
Sample preparation: 500 μL Milk + 2 g C18 material + 10 μL MeOH + 10 μL 12.5 μg/mL sulfamerazine in MeOH, let stand for 1 min, grind with a glass pestle until homogeneous, place in a 10 mL syringe barrel plugged with filter paper, place filter paper on top, compress to 4.5 mL with a plunger, restrict column outlet with a 100 μL pipette tip, wash with 8 mL hexane, remove excess hexane with positive pressure, elute with 8 mL dichloromethane, elute excess dichloromethane with positive pressure. Evaporate the eluate under a stream of nitrogen, dissolve the residue in 100 μL MeOH and 400 μL 17 mM orthophosphoric acid, sonicate for 5-10 min, centrifuge at 13600 g for 5 min, filter supernatant (0.45 μm), inject a 20 μL aliquot. (C18 material was Analytichem 40 μm 18% load endcapped. Add 22 g to a 50 mL syringe barrel wash with 2 column volumes of hexane, 2 volumes of dichloromethane, and 2 volumes of MeOH, vacuum aspirate until dry.)

HPLC VARIABLES
Column: 75 × 4 3 μm Supelcosil LC-18
Mobile phase: MeCN:17 mM orthophosphoric acid 10:90
Column temperature: 45
Flow rate: 1 for 5 min then 2 for remainder of run
Injection volume: 20
Detector: UV 270

CHROMATOGRAM
Retention time: 8.8
Internal standard: sulfamerazine (3)
Limit of detection: 62.5 ng/mL

OTHER SUBSTANCES
Simultaneous: sulfamethoxazole, sulfanilamide, sulfathiazole, sulfadiazine, sulfamethazine, sulfadimethoxine

KEY WORDS
matrix solid-phase dispersion

REFERENCE
Long,A.R.; Short,C.R.; Barker,S.A. Method for the isolation and liquid chromatographic determination of eight sulfonamides in milk, *J.Chromatogr.*, **1990**, *502*, 87–94.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 20:1.5:0.5:78
Flow rate: 1.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 15

OTHER SUBSTANCES
Simultaneous: sulfanilic acid, sulfanilamide, sulfapyridine, sulfamerazine, sulfadiazine, sulfamethizole, sulfamethazine, sulfamethoxazole, sulfachlorpyridine

REFERENCE
Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4 OmniPac PCX-500 (Dionex)
Mobile phase: Gradient. A was MeCN:110 mM perchloric acid and 20 mM sodium acetate 27:73. B was MeCN:110 mM perchloric acid and 100 mM sodium acetate 50:50. A:B from 100:0 to 0:100 over 5 min, then re-equilibrate.
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 5.6

OTHER SUBSTANCES
Simultaneous: sulfamerazine, sulfamethazine, sulfamethizole, sulfamethoxazole, sulfanilamide, sulfanilic acid, sulfathiazole

REFERENCE
Slingsby,R.W.; Rey,M. Determination of pharmaceuticals by multi-phase chromatography: Combined reversed phase and ion exchange in one column, *J.Liq.Chromatogr.*, **1990**, *13*, 107–134.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in MeOH:water 25:75, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 250 × 2.1 5 μm 201TP (Vydac)
Mobile phase: Gradient. A was MeCN containing 0.1% trifluoroacetic acid. B was water containing 0.1% trifluoroacetic acid. A:B from 5:95 to 40:60 over 20 min.
Flow rate: 0.2
Injection volume: 5
Detector: UV 270 or MS, Sciex API III triple quadrupole, IonSpray interface

CHROMATOGRAM
Retention time: 15.57

OTHER SUBSTANCES
Simultaneous: phthalylsulfathiazole, succinylsulfathiazole, sulfabenzamide, sulfacetamide, sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfaguanidine, sulfamerazine, sulfameter, sulfamethazine, sulfamethizole, sulfamethoxazole, sulfamethoxypyridazine, sulfamoxole, sulfanilamide, sulfapyridine, sulfaquinoxaline, sulfathiazole, sulfisomidine

REFERENCE

Pleasance,S.; Blay,P.; Quilliam,M.A.; O'Hara,G. Determination of sulfonamides by liquid chromatography, ultraviolet diode array detection and ion-spray tandem mass spectrometry with application to cultured salmon flesh, *J.Chromatogr.*, **1991**, *558*, 155–173.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a solution in mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 Nucleosil 5C18
Mobile phase: MeCN:10 mM pH 5.6 phosphate buffer 8:92
Flow rate: 1
Injection volume: 20
Detector: UV 270

CHROMATOGRAM

Retention time: 20.2

OTHER SUBSTANCES

Simultaneous: N-acetylsulfisomidine, sulfachloropyridazine, sulfadimethoxine, sulfadoxine, sulfamethazine (sulfadimidine), sulfamethoxypyridazine, sulfamonomethoxine, sulfisomidine

REFERENCE

Nishikawa,M.; Takahashi,Y.; Ishihara,Y. High performance liquid chromatographic determination of sulfisomidine and N4-acetylsulisomidine in swine tissues, *J.Liq.Chromatogr.*, **1993**, *16*, 4031–4047.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 300 × 0.35 5 μm Vydac IDI-TP C18 TMS capped
Mobile phase: Gradient. MeOH:buffer 0:100 at start of run, to 10:90 after injection (step gradient), to 12:88 over 30 min, to 18:82 over 5 min, to 30:70 over 5 min. (Buffer was 1 mM pH 2.72 phosphate buffer
Column temperature: 30
Flow rate: 0.006
Injection volume: 1
Detector: UV 270

CHROMATOGRAM

Retention time: 51

OTHER SUBSTANCES

Simultaneous: diaveridine, phthalyl sulfathiazole, pyrimethamine, succinyl sulfathiazole, sulfabenzamide, sulfacetamide, sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfaguanidine, sulfamerazine, sulfameter, sulfamethazine, sulfamethizole, sulfamethoxazole, sulfamethoxypyridazine, sulfamoxole, sulfanilamide, sulfanilic acid, sulfapyridine, sulfaquinoxaline, sulfathiazole, sulfisomidine, trimethoprim

KEY WORDS

capillary HPLC

REFERENCE

Ricci,M.C.; Cross,R.F. High-performance liquid chromatographic analyses of sulphonamides and dihydrofolate reductase inhibitors. I. Separations in methanol-modified solutions, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 365–381.

SAMPLE

Matrix: solutions

HPLC VARIABLES
Column: 300 × 0.35 5 μm Vydac IDI-TP C18 TMS capped
Mobile phase: Gradient. MeCN:MeOH:buffer 0:0:100 at start of run, to 0:5:95 after injection (step gradient), to 0:8:92 over 7 min, to 6:0:94 (step gradient), maintain at 6:0:94 for 14 min, to 0:16:84 over 5 min, to 0:18:82 over 5 min, to 0:30:70 over 5 min. (Buffer was 1 mM pH 2.72 phosphate buffer.)
Column temperature: 30
Flow rate: 0.006
Injection volume: 1
Detector: UV 270

CHROMATOGRAM
Retention time: 46

OTHER SUBSTANCES
Simultaneous: diaveridine, phthalyl sulfathiazole, pyrimethamine, succinyl sulfathiazole, sulfabenzamide, sulfacetamide, sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfaguanidine, sulfamerazine, sulfameter, sulfamethazine, sulfamethizole, sulfamethoxazole, sulfamethoxypyridazine, sulfamoxole, sulfanilamide, sulfanilic acid, sulfapyridine, sulfaquinoxaline, sulfathiazole, sulfisomidine, trimethoprim

KEY WORDS
capillary HPLC

REFERENCE
Ricci,M.C.; Cross,R.F. High performance liquid chromatographic analyses of sulphonamides and dihydrofolate reductase inhibitors. II. Separations in acetonitrile modified solutions, ternary gradient studies & flow programming, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 547–564.

Sulfur

Molecular formula: S
Molecular weight: 32.06
CAS Registry No.: 7704-34-9
Merck Index: 9142

SAMPLE
Matrix: formulations
Sample preparation: Add a 200 mg tablet containing 6 mg captan and 5 mg sulfur to 5 mL carbon disulfide, extract the solid residue five times with 5 mL carbon disulfide, combine the extracts and evaporate to constant weight. Dissolve the residue in 2 mL carbon disulfide, make up to 10 mL with MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 10 μm Perkin-Elmer C8
Mobile phase: MeOH:water 90:10
Flow rate: 1
Injection volume: 20
Detector: UV 254

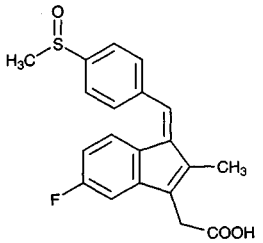
CHROMATOGRAM
Retention time: 7

OTHER SUBSTANCES
Simultaneous: captan

KEY WORDS
tablets

REFERENCE
Fedeli,G.; Moltrasio,D.; Aleotti,M.; Gazzani,G. High-performance liquid chromatographic determination of sulphur and captan in a mixture, *J.Chromatogr.*, **1988**, *447*, 263–267.

Sulindac



Molecular formula: $C_{20}H_{17}FO_3S$
Molecular weight: 356.42
CAS Registry No.: 38194-50-2
Merck Index: 9155
Lednicer No.: 2 210

SAMPLE
Matrix: bile, blood
Sample preparation: Plasma, urine, or bile + 2 mL 1 M HCl + 5 mL chlorobutane:1,2-dichloroethane 80:20, extract, centrifuge. Remove the organic layer and add it to 400 µL 100 mM NaOH, shake for 5 min, centrifuge, inject a 200 µL aliquot of the aqueous layer.

HPLC VARIABLES
Column: µBondapak
Mobile phase: MeCN:200 mM pH 3.5 ammonium phosphate buffer 50:50
Flow rate: 1.6
Injection volume: 200
Detector: UV 254

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; urine; rabbit

REFERENCE
Strong,H.A.; Renwick,A.G.; George,C.F. The site of reduction of sulphinpyrazone in the rabbit, *Xenobiotica*, **1984**, *14*, 815–826.

SAMPLE
Matrix: bile, blood, gastric contents, tissue, urine
Sample preparation: 100 µL Whole blood, bile, liver homogenate, urine, or gastric contents + 100 µL 1 mg/mL ketoprofen + 1 mL 3 M pH 1.5 phosphate buffer + 3 mL chloroform, vortex, rotate for 10 min, centrifuge for 10 min. Remove the organic layer and add it to 1 mL 50 mM NaOH, mix for 5 min, centrifuge for 5 min. Remove the aqueous layer and neutralize it with 1 mL 50 mM phosphoric acid, inject an aliquot.

HPLC VARIABLES
Column: 50 mm long C18
Mobile phase: MeCN:350 mM acetic acid 35:65
Flow rate: 2
Detector: UV 313

CHROMATOGRAM
Internal standard: ketoprofen

KEY WORDS
whole blood; liver